Final Report for Project # 20040609

DISTRIBUTION, SEASONALITY AND PERSISTENCE OF MYCOBACTERIUM PARATUBERCULOSIS (JOHNE'S DISEASE) IN THE ENVIRONMENT OF COW-CALF FARMS IN WESTERN CANADA

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SUMMARY:

Six Johne's disease positive cow-calf herds from Saskatchewan were studied quarterly for one year in order to: 1) identify the environmental areas including water sources on a cow-calf farm that become contaminated with Mycobacterium avium subspecies paratuberculosis, Map, and how long these areas remain infectious, 2) perform a preliminary assessment of the potential of environmental sampling as an alternative to herd serological and pooled fecal testing on cow calf farms, 3) establish if there are seasonal influences that affect the environmental contamination of these farms, 4) formulate informed recommendations regarding the environmental and water quality concerns for the control of Johne's disease on cow-calf farms in Saskatchewan. 33.3% of these herds were identified as positive for Map by both the ELISA and environmental sampling methods and 66.7% of these herds were identified as positive for Map by the pooled fecal culture method. 0.5% of the environmental samples collected were positive for Map on culture with no significant difference found depending on farm prevalence or season. The positive samples were collected from a dugout and a calving pen. Map was not found to survive in the environment for up to 5 months. At this time, the environments of cow-calf herds with a history of Johne's disease in Saskatchewan are not significantly contaminated with Map and sampling the environment does not appear to be an adeqaute method to use as an alternative to traditional herd test methods.

INTRODUCTION:

Johne's Disease, caused by Mycobacterium avium subspecies paratuberculosis, Map, is a progressive and debilitating disease of livestock affecting beef cattle in Western Canada. It causes a profuse, untreatable diarrhea with high levels of bacterial shedding, dramatic weight loss, and eventually death. The primary modes of transmission include either direct exposure through the fecal-oral route, through contact with a contaminated environment or through consumption of infectious colostrum or milk (Giese and Ahrens, 2000). Calves from cow-calf herds, unlike dairy herds, cannot be isolated immediately after birth and so control efforts must focus on culling infected cattle and reducing exposure to the bacteria from environmental sources. Environmental contamination on cow-calf herds has not yet been studied but is vital for the successful management of this disease. Map has been shown to be able to survive well in various environmental conditions (Whittington et al., 2004). The level of environmental contamination is dependent on the persistence of the bacteria in the environment as well as the degree of recontamination from cattle or other potential carrier species. Multiple regions around the world and within Canada have proactively developed Johne's control programs due to the concern that Johne's disease may be involved with Crohn's disease in humans (Chiodini et al., 1984; Collins and Manning, 1995; Hermon-Taylor, 2000; Manning, 2001). The process of controlling Johne's disease involves repeated testing of the herd, culling affected animals, and cleaning up the environment prior to releasing new animals onto the previously contaminated areas. This is an expensive and difficult process. If the environmental contamination persists, control may never be achieved which equates to

economic loss due not only to lost production but also the cost of repeatedly testing the herd, lost use of contaminated areas, and potential lost markets for cattle. Environmental testing has been suggested as an alternative to the traditional herd test methods for the dairy industry and could reduce the cost of testing a herd if it can replace individual animal testing (Raizman et al., 2004).

Water quality has always been important in disease control. Contaminated water acts as a potential source of infection for cattle, other animals and humans as well. Contaminated water could potentially move downstream as well as into the groundwater causing further environmental and health concerns.

The objectives of this study are to:

1. Identify the environmental areas including water sources on a cow-calf farm that become contaminated with Map and how long these areas remain infectious.
2. Perform a preliminary assessment of the potential of environmental sampling as an alternative to herd serological and pooled fecal culture on cow-calf farms.
3. Establish if there are seasonal influences that affect the environmental contamination of these farms.
4. Formulate informed recommendations regarding the environmental, and water quality concerns for the control of Johne's disease on cow calf farms in Saskatchewan.

METHODS:

Starting in the summer of 2005, approximately 30 beef cow-calf herds from Western Canada were identified as being actively infected with Johne's disease. Herds were recruited through contacts with local veterinarians and producers were asked if they wished to participate in the study. An actively infected herd was considered a herd that had had clinical cases of Johne's disease diagnosed by their veterinarian within the last 2 years and at least one positive laboratory test confirmation. Herds were selected due to a willingness to participate and accessability of cattle for sampling. The selected herds were tested to determine the prevalence of Map infections. 6 herds were selected for the Distribution and Seasonality portion of the study and 1 herd was selected for the Persistence portion of the study..

Distribution and Seasonality Portion of the Study:

Six farms were selected for a one-year study examining the relationship between environmental contamination and Johne's prevalence within a cow calf herd. In these 6 farms, blood samples were taken from approx 100 cattle greater than 2 years of age in the fall of 2005, and were tested with a ParaChek ELISA for Map titres by the Animal Health Monitoring Lab of the Abbotsford Agriculture Centre. Pooled fecal cultures (five cattle per culture) were also done on these herds to enable comparison with both the serological and environmental sampling results. Four rounds of environmental sampling were conducted over the span of one year (one round of sampling every three months). To determine the distribution of environmental contamination, 15 environmental samples were taken per sampling round from similar sites at each selected farm including calving

areas, feeding areas, and water samples. Water samples were collected from livestock waterers, dugouts, ditches, and streams when present. Water sources and runoff areas were cultured to determine their significance to both on-farm transmission and to broader environmental contamination leading to the potential spread to locations downstream. Each non-water environmental sample was collected by combining approximately 15ml of sample from 4 locations per site for a total of approximately 60ml of combined sample per site. Water samples consisted of 500ml of water collected from a site. This water was centrifuged at 1000g for 30 minutes and the resulting sediment was collected and sent for culture. Biofilm samples were collected at water sources by taking a clean 4 x 4 guaze and wiping the entire interior surface of the waterer. Environmental and pooled fecal samples were frozen until being submitted for culture after each round at the Animal Health Monitoring Lab in Abbotsford, British Columbia where they were stored frozen until culture. The modified BACTEC 12B culture method was utilized for detecting Map from these samples. All positive cultures were tested using the IS900 polymerase chain reaction (PCR) technique which uses the presence of a Map specific gene to confirm the positive result.

Persistence Portion of the Study:

One herd was selected for the persistence portion of this study due to having environmental areas known to be contaminated with detectable Map. This herd was not one of the 6 selected herds studied above. The initial plan was to use sites identified as Map positive in the first round of sampling. This was not possible due to the low level of contamination found on the initial 6 selected herds. Three areas were identified as

contaminated with Map on this farm and were re-cultured monthly once these areas were confirmed to be culture positive for Map. This was done to determine how long Map could survive in these relatively natural conditions. Two samples per site were taken regularly until they were culture negative for two consecutive sampling periods.

RESULTS:

6/6 (100%) of herds selected completed all 4 rounds of the required sampling and were included in the analysis. Of these 6 herds, 2 (33.3%; 95%CI 0-74.7) were identified as Johne's positive by having at least 2 positive serological tests and 4 (66.7%; 95%CI 25.3-100) of 6 were identified as Johne's positive by having at least one positive pooled fecal culture. Individual herds had between 0.5% and 15.3% of the individual cattle test positive on the ELISA and between 0% and 52.0% of the pooled fecal cultures test positive. Table 1 shows the summary of the farm test results.

1/150 (0.7%; 95%CI 0-2.0) of the water samples made up of: 48 samples from waterers, 24 biofilm samples, 55 dugout samples, and 23 farm drainage samples were positive for Map. The positive culture was cultured from a dugout sample collected in the summer round of sampling from herd #6. 1/268 (0.4%; 95%CI 0-1.1) of the environmental samples (non-water) were positive for Map. The single positive culture was from a calving pen sample collected in the fall round of sampling from herd #4. Map was not detected in the any of the samples from the remaining sites. Table 2 summaries the culture results of the environmental samples grouped according to the season of sample collection.

Two (33.3%; 95%CI 0-74.7) of the 6 herds were identified as Johne's positive through environmental sampling. The three alternative herd tests had varying agreement. When the ELISA test results were compared to the pooled fecal culture a kappa of -0.20 was

calculated. This indicates that the agreement between theses two tests was less than what would be expected due to chance alone. When comparing the environmental testing method to the pooled fecal culture the kappa was calculated to be 0.40 which is considered to be moderate agreement. The kappa calculated when comparing the environmental sampling with the ELISA testing was 0.25 which is considered fair agreement. Table 3 compares the ability of the three separate herd tests to identify a farm with a history of clinical Johne's disease as a positive herd.

For the persistence study, 3 sites were identified as culture positive on a cow-calf farm in Saskatchewan not included in the rest of the study. These sites were all negative on culture when sampled again 5 and 6 months after the initial sampling.

CONCLUSIONS AND RECOMMENDATIONS:

The level of environmental contamination was very low on the farms participating in this study with only 2/418 samples collected being positive for Map. This may be due to a small farm sample size or missing the contaminated sites but is likely due to a combination of multiple factors such as a relatively low prevalence of disease in this industry and the extensive management practices of cow-calf farms including an relatively low cattle density. In this study the only sites to be culture positive were a dugout sample and a calving pen sample. The positive dugout sample may be a concern as Map is known to survive for long periods of time in water and this could potentially be a source of transmission to other animals sharing that water source. The positive calving pen sample may potentially be significant if young calves remain in the pen for a significant period of time as these young calves have an increased susceptibility (Manning and Collins, 2001). While no other sites were positive in this sample further research on farms with a higher prevalence of disease could determine which other sites should be considered high risk for contamination.

There were not enough positive environmental sites in this project to study the seasonal effects. Only samples from summer and fall had any positive samples but this is not a significant finding at this level. It is hypothesized by the authors of this study that sampling during the calving season on more heavily infected farms may pose an increased risk of contamination due to higher cattle densities and peripartum stress factors but this hypothesis was not supported by the results of this study.

The low level of contamination of environmental sites made the interpretation of the results of the persistence portion of this study difficult. This portion of the study did not find any Map surviving up to 5 months. This suggests that Map may not survive the natural conditions and climate present at these sites for durations up to a year in length as has been reported for other environments in previous papers (Whittington et al., 2004). This issue should be studied further through an experimental field study to ensure site contamination. This would enable samples to be collected regularly from the time of contamination until the sites are no longer culture positive. This would allow for more specific results in the first 5 months of the study that were not possible in this pilot project.

The low level of contaminated samples and the low ability of the environmental sampling to identify herds as positive for Map suggest that environmental sampling as done in this project is not likely an adequate alternative herd test. While the results of environmental sampling had moderate agreement (kappa = 0.40) with fecal pool sampling, it only had fair agreement (kappa = 0.25) with the results of serological sampling using the ELISA in this study. The poor level of agreement between fecal pooled sampling and the ELISA is remarkable however some of this is likely due to the stage of disease that the individual cattle were in at the time of sampling. Both fecal pooled sampling and the ELISA identified herds as positive that were not identified by any other test as seen in Table 3. All herds had a recent history of clinical disease prior to the start of this study and it was therefore expected that each herd should test positive by at least one testing method. The level of environmental contamination on cow-calf farms in Saskatchewan is

quite low as shown by only 2 of 6 herds in this study having any positive sites detected. While reducing the cost of testing, using environmental samples as done in this study is not sensitive enough as a herd test to be used in place of traditional herd test methods. The infection levels on these farms were low and may be the reason that Map was not able to be detected more frequently in the environmental sampling. Further study is required including more herds and focused on sites with higher cattle densities to definitively conclude whether or not environmental testing on cow-calf herds could have any value as an alternate herd test for Johne's disease.

The results of this study show that the level of environmental contamination of Map on cow-calf herds in Saskatchewan is very low at this time. This suggests that efforts to control this disease should be focussed on other management factors that can reduce the exposure of susceptible animals to Map such an not keeping infected animals on farm and reducing the movement of potentially infected animals onto the farm. The cow-calf industry has an opportunity to control this disease now before the prevalence of the disease increases in the population and before environmental contamination becomes a more significant challenge.

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APPENDICIES:

Table 1. Summary of farm test results

Herd #	Serology Results	Pooled Fecal Culture	Environmental Sampling
1	1/111	1/23	0/60
2	1/184	0/20	0/64
3	2/68	0/14	0/66
4	1/84	1/17	0/82
5	19/124	13/25	1/77
6	1/105	1/21	1/69

Table 2. Culture results of four rounds of environmental sampling on all farms

	Non Water Source Environmental Samples	Water Source Samples	All Environmental Samples
Fall	1/58	0/41	1/99
Winter	0/71	0/35	0/106
Spring	0/74	0/40	0/114
Summer	0/65	1/34	1/99
Total	1/268	1/150	2/418

Table 3. Comparison of the results of three alternative herd test methods

Herd #	Test Method		
	Pooled Fecal Culture*	Serum ELISA**	Environmental Testing***
1	+	-	-
2	-	-	-
3	-	+	-
4	+	-	-
5	+	+	+
6	+	-	+

* + herd = 1 positive pool (5 samples/pool)

** + herd = >1 positive serological test

*** + herd = 1 culture positive environmental sample

OTHER:

Results from this study have been combined with results from concurrent research and presented at the Animal Determinants of Emerging Disease Rounds. These combined results were submitted as an abstract to the American Association of Bovine Practitioners to be considered as a presentation at the AABP Conference in 2007. The combined results will also be submitted for publication in a peer reveiwed journal as well as published as part of a Ph D thesis for Dr. Dale Douma. The final expense statement is in the process of being generated and will be submitted as soon as completed.